

## **Microbial Influenced Corrosion on Aluminium by *Pseudomonas fluorescens* in different saline water**

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**Abstract :** Microbialinfluenced corrosion is a major issue for some industries. The ooccurrence of corrosion deposits were related to the activities of microorganism. *Pseudomonas sp.* could affect the rate of metal corrosion due to this bacteria can produce polymer during biofilm formation. The aims of this research were to determine the corrosion rate on aluminium alloy (AA)6063 in three of salinity (33, 35 and 37‰) and to determine MIC rate was caused by *Pseudomonas fluorescens*. Aluminium 6063 specimens were exposed to seawater media containing the aerobic bacterium *P.fluorescens*. The weight loss method was used in this research. The results showed the corrosion rate, without bacteria, on AA6063after immersion in salinity of 33, 35 and 37‰ at day 28 were 0.5383, 0.6553 and 0.7021 mm/year, respectively. The higher salinity can cause heavy pitting and thus cause pitting corrosion on aluminimum. The highest MIC rate on AA6063 was 1.1701 mm/year at day 28 in salinity of 37‰ by *P. fluorescens*. Meanwhile, the MIC at salinity of 33 and 35‰ were 0.7021 and 1.0765 mm/year at similar day respectively. Based on macrostructure and microstructure, it depicts pitting corrosion on AA6063 can be accelerated by presence of *P.fluorescens*. In conclusion, *P. fluorescens* has a role to increase of pitting corrosion rate on AA 6063.

**Keywords :** bacteria, corrosion, MIC rate, marine environment, salinity.

### **Introduction**

Corrosion is the destructive attack of a metal by chemical or electrochemical reaction with its environment<sup>1</sup>. Microbial Influenced Corrosion (MIC) is a mechanism referring to the corrosion of metal surfaces influenced by the physiological processes of microorganisms<sup>2</sup>. Microbially influenced corrosion also is known as bio-corrosion<sup>3</sup>. According to Shi et al.<sup>4</sup>, MIC could cause corrosion on stainless steel, carbon steel, aluminium, zinc and copper alloys. The microorganisms associated with biocorrosion are widely occurred in freshwater, sea water, industrial systems and storage tanks, among others and may influence the spread of all types of metal corrosion. Microbial corrosion can accelerate most forms of corrosion; including uniform corrosion, pitting corrosion, crevice corrosion, galvanic corrosion, intergranular corrosion, dealloying, and stress corrosion cracking<sup>5</sup>. MIC is rarely linked to a single mechanism or to a single species of microorganisms<sup>6</sup>.

According to Beech IB and Coutinho<sup>7</sup>, the main types of bacteria associated with metals in terrestrial and aquatic habitats are the extracellular polymeric substances (EPS)-producing bacteria, acid-producing bacteria, sulfur oxidizing bacteria, iron precipitating bacteria and sulfate reducing bacteria (SRB). Biofilm forming bacteria and SRB may mediate interactions between metal surfaces and the liquid environment, leading to major modifications of the metal-solution interface<sup>6</sup>. Microbial activity within biofilms formed on surfaces of metallic materials can considerably modify the chemistry of any protective layers, leading to either acceleration or inhibition of biocorrosion<sup>8,9</sup>. Thomas et al.<sup>10</sup> suggested that the presence of a biofilm may act as a diffusional barrier to the corrosive substances and prevent them from reaching the metal surface.

Biofilms consist of microbial cells, their EPS, which facilitate irreversible attachment of cells to the surface, inorganic precipitates derived from the bulk aqueous phase and/or corrosion products of the metal substratum<sup>11</sup>. EPS was formed by proteins, polysaccharides, lipids and nucleic acids, protects microbial cells against metal ions, biocidal action and helps to trap other microbial species, thereby contributing to increased thickness of biofilm<sup>3</sup>. Bacteria secrete EPS to envelope and to anchor them to the substrate there by altering the local surface chemistry which can stimulate further growth and settlement of macro organisms<sup>12</sup>.

*Pseudomonas* is the main genus of EPS producer<sup>3</sup>. *Pseudomonas* (and other aerobic bacteria) have been shown to both increase and decrease the rate of metal corrosion through polymer production during biofilm formation<sup>13,14</sup>. *Pseudomonas* sp. 200 was known to accelerate corrosion with increasing growth rate<sup>15</sup>. *Pseudomonas* sp that isolated from products of corrosion of diesel pipelines and metal surfaces immersed in fresh water and sea water could produce EPS that favors the formation of biofilm<sup>3</sup>.

Salinity is one of factors that affect the corrosion rate in seawater<sup>16</sup>. Salinity affects two factors involved in corrosion rate, namely conductance of the electrolyte and chlorine content. Salinity is the total amount of dissolved salts in seawater in grams/kilograms or parts per thousand. Average ocean salinity is 35, but salinity ranges from 33 to 37 in the surface waters of the open ocean. Brackish water has a salinity of less than 25 while hypersaline water has a salinity greater than about 40<sup>17</sup>. Salinity is usually determined either by conductivity measurements or from the chlorinity. This is nearly equal to the mass of chloride in the seawater sample. Chlorinity (‰) is related to salinity *S* (‰) based on the equation = 1.80655 x Cl (‰)<sup>18</sup>.

Aluminum alloys play a vital role in engineering field as it possesses high specific strength and density<sup>19</sup>. Aluminium and aluminium alloys have emerged as alternate materials in aerospace and in some chemical processing industries<sup>20</sup>. AA6063 has been widely used in the fabrication of lightweight structures requiring high strength-to-weight ratios<sup>21</sup>. MIC could cause corrosion on aluminium alloy. Shi et al.<sup>4</sup> The objective of the study is to determine corrosion rate on AA6063 in marine environment with variable of salinity (33, 35 and 37‰). Second aim is to determine bio-corrosion rate on AA6063 in different saline water by *Pseudomonas fluorescens*.

## Materials and Methods

### Preparation of bacteria

This research was carried out using species bacteria of *Pseudomonas fluorescens*. The pure culture of bacteria was inoculated onto nutrient agar (NA) media using streak plate technique<sup>22</sup>. The age of bacteria for MIC test was 24 h. After that, one colony of bacteria was transferred to nutrient broth (NB), and kept in shaker incubator at 150 rpm and room temperature, 33 °C for 24 h<sup>23</sup>. The suspension of bacteria in Optical Density (OD) of 1 was ready to be used in bio-corrosion test.

### Preparation of specimen for corrosion test

Specimens test for corrosion was AA6063 with area of  $\pm 2 \text{ cm}^2$ . Specimens were polished using a grade paper. Composition of AA6063 was determined using Glow Discharge Profiler (Model-Horiba Jobin Yvon, Perancis). Table 1 showed the composition of specimen test, AA6063.

**Table 1. Material composition of AA6063**

Elements	AA6063 ( % weight)
Si	0.531 ± 0.157
Fe	0.251 ± 0.009
Cu	0.017 ± 0.003
Mg	0.440 ± 0.008
Mn	0
Cr	0.005 ± 0.001
Zn	0.284 ± 0.345
Ti	0.026 ± 0.006
Co	0.008 ± 0.007
Al	balance

**Preparation of medium for corrosion test**

Artificial seawater was be used in this research and the formula of artificial seawater based on ASTM standard D1 141-90<sup>24</sup>. Table 2 showed the chemical composition of artificial seawater.

**Table 2 Chemical composition of artificial sea water<sup>A,B</sup>(ASTM D1 141-90) for 35‰**

Solution	Concentration, g/L
NaCl	24.53
MgCl <sub>2</sub>	5.20
Na <sub>2</sub> SO <sub>4</sub>	4.09
CaCl <sub>2</sub>	1.16
KCl	0.695
NaHCO <sub>3</sub>	0.201
KBr	0.101
H <sub>3</sub> BO <sub>3</sub>	0.027
SrCl <sub>2</sub>	0.025
NaF	0.003
Ba (NO <sub>3</sub> ) <sub>2</sub>	0.0000994
Mn(NO <sub>3</sub> ) <sub>2</sub>	0.0000340
Cu(NO <sub>3</sub> ) <sub>2</sub>	0.0000308
Zn(NO <sub>3</sub> ) <sub>2</sub>	0.0000096
Pb(NO <sub>3</sub> ) <sub>2</sub>	0.0000066
AgNO <sub>3</sub>	0.00000049

<sup>A</sup>Chlorinity = 19.38

<sup>B</sup> pH (after suitable with 0.1 N sodium hydroxide)= 8.2

Table 2 showed salinity condition of 35 (35‰), so that for finding salinity of 33 (33‰), can use this calculation:

$$NaCl \text{ in salinity } 33 \text{ ‰} = \frac{0.33}{0.35} \times 24.53 \text{ g/L}$$

$$= 23.13 \text{ g/L}$$

**Corrosion test**

Test of MIC was conducted according to Pratikno and Titah<sup>23</sup>. Amount of 5% (v/v) or around 300,000,000 CFU/mL of *P. fluorescens* was added in corrosion test solution.

## Corrosion Rate Calculation

The corrosion rate and bio-corrosion rate in millimeter per year (mm/year) were calculated from weight of material loss during test. Although the method is the conventional method but the results are very accurate<sup>25</sup>. The formula of weight loss methods is<sup>26,27</sup>:

$$\text{mm/year} = 12 \times \frac{(7290 W)}{(A.t.d)}$$

t=time of exposure (hours)

A=area (cm<sup>2</sup>)

W=weight loss (gram)

d=density (gram/cm<sup>3</sup>)

For Al, density=2,67 gram/cm<sup>3</sup>

## Macrostructure and Microstructure Observation

Preparation of specimen was conducted based on ASTM G 163<sup>28</sup>. The specimen should be spilled with a Keller reagent for 5 seconds before it was observed on macro and microstructure. After that, the specimen was rinsed using deionization water and it was dried. Keller reagent was prepared based on the ASM Handbook<sup>29</sup>, which is to make 200 ml reagent Keller needed:

- 2 ml hydrofluoric concentrated acids, HF (density 40%),
- 3 ml of concentrated hydrochloric acid, HCl (density 37%),
- 5 ml of concentrated nitric acid, HNO<sub>3</sub> (density 70%),
- 190 ml of distilled water.

Macrostructure observation on the specimen was conducted using Olympus CX41 (Japan) in 40X magnification. Meanwhile, microstructure observation was carried out using optical microscope AxioCam Carl Zeiss Vision 4.4 (Germany).

## Results and Discussion

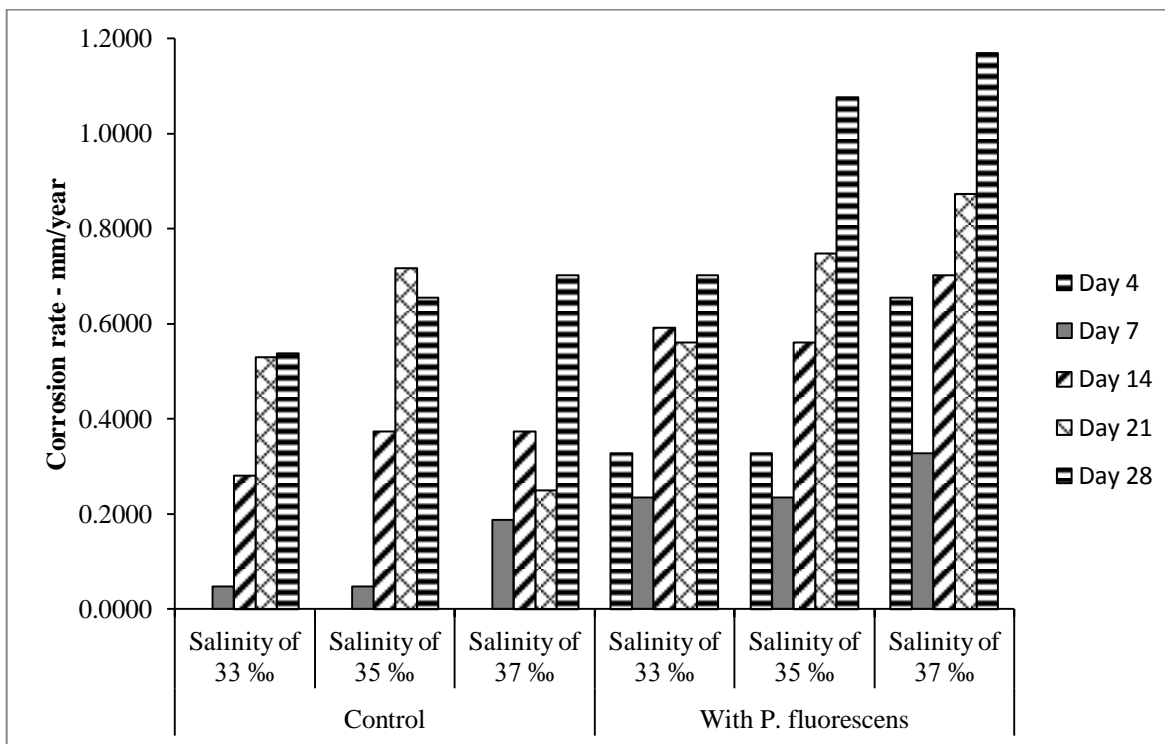
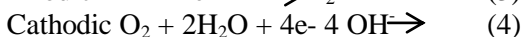
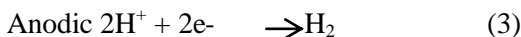
### Corrosion Rate

Based on the results, Figure 1 showed high salinity caused value of corrosion rate increasing. The corrosion rate of AA6063 as a control after immersion in salinity of 33‰, 35‰ and 37‰ at day 28 were 0.5383, 0.6553 and 0.7021 mm/year, respectively. Higher salinity means high concentration of chloride ions. The ion of chloride increased during salinity increased. Thus higher salinity can cause heavy pitting and thus damage the metal. According to Paul<sup>30</sup>, chloride ion increases the corrosion rate. Chloride ions can easily penetrate through the passive oxide film and cause breakdown of the passive layer<sup>31</sup>. Chloride ions is the major contributors in the post recovery corrosion from the pits in the metal surface<sup>32</sup> and it could cause pitting corrosion. Hagioglu et al.<sup>33</sup> reported that aluminium has very low resistance to a corrosive environment (30‰NaCl) in a static regime, and corrosion processes take place on the aluminium surface. Based on Al-Tai<sup>34</sup>, the corrosion rate of aluminum alloy were 0.0292 and 0.0474 mm/year respectively at day 15 and 30 in 20‰ of NaCl or brackish water. The range of aluminium corrosion rate at coastal area were <0.01 – 0.125 mm/year<sup>35</sup>.

According to Gujarathi<sup>16</sup>, chloride ions are responsible for pitting of aluminium alloy 2024. Pitting is a form of corrosion resulting in the creation of small holes in the substrate<sup>2</sup>. Corrosion pitting is the result of interactions between an anode and a cathode. A media containing electrolytes enables ions to move from the anode to the cathode causing losses in the anode leading to the formation of small pits. Pitting is a highly localized type of corrosion in the presence of aggressive chloride ions<sup>36</sup>. Pits are initiated by chloride attack at weak sites in the metal oxide; the pits propagate according to the two following reaction<sup>34,37</sup>.



While hydrogen evolution and oxygen reduction are the important reduction processes at anode and cathodes.



**Figure 1 Corrosion Rate on AA6063**

The high MIC rate on AA 6063 was 1.1701 mm/year at day 28 in salinity of 37‰ by *P. fluorescens* (Figure 1). Meanwhile, the MIC at salinity of 33 and 35‰ were 0.7021 and 1.0765 mm/year at similar day respectively. It was indicating that salinity affected the MIC rate on AA6063. The higher salinity can cause heavy pitting corrosion. The MIC rate on AA6063 showed higher value than corrosion rate in control (without bacteria, only salinity). It means that presence of *P. fluorescens* for a given period of time could be accelerated the corrosion rate. In MIC, the anode or substratum is losing ions to the microorganism so it can maintain its respiratory pathways. In addition, the formation of biofilms enables high concentration of hydrogen ions leading to a highly acidic environment<sup>2</sup>. This environment is thus more susceptible to continued corrosion and pitting. The MIC rate by *P. fluorescens* showed value up to 1 mm/year and it increased by one point six-fold compared with corrosion rate without bacteria. Al-Thai<sup>34</sup> reported that the role of *P. aeruginosa* bacteria in accelerating pitting corrosion on aluminum alloy in the chloride medium. Based on Al-Tai<sup>34</sup>, the corrosion rate on aluminium alloy in 20‰ of NaCl inoculated with 4 mL of *P. aeruginosa* bacteria or 0.5% (v/v) of bacteria were 0.0949 and 0.0876 mm/year after 15 and 30 days, respectively.

### Surface Analysis

Based on visual observation (Figure 2), the corrosion occurred on both sides of the test specimen. It looked a brownish color on the surface of the AA6063 specimens in all salinity of 33, 35 and 37‰ after immersed in artificial sea water. Brownish color seemed more and it was more spread in specimens in saline solution with the *P. fluorescens* when it compared with the corrosion occurred in only saline solution.

### Macrostructure Analysis

Figure 3 showed macrostructure of AA6063 specimen in 40X magnification. The corrosion on only saline solution occurred in all salinity. The corrosion appeared on surface of the specimen at salinity of 33 ‰ still looked flat and smooth (Figure 3a), but at salinity of 37 ‰ control was visible white spots of corrosion on the surface of the specimen (Figure 3c). It showed the acceleration of the corrosion of the surface specimen addition of *P. fluorescens* in marine water replacement solution. The corrosion on surface of specimen with the addition of *P. fluorescens* bacteria after 28 days showed that corrosion occurred in all salinity (Figure 3d,e,f). The

salinity was higher so that the macrostructure of corrosion was higher too. It looked white spots on the all surface of the specimen.

### Microstructure Analysis

Based on microsturcture observation (Figure 4), the surface of AA6063 with *P. fluorescens* revealed a large quantity of corrosion products and more corrosion pits were evident in the presence of *P. fluorescens*. Pitting corrosion of AA6063 in all salinity. It indicates that the ability of *P. fluorescens* to cause corrosion and form defects, cracking, pits and holes result from corrosion process which had covered the surface of AA6063. According to Al-Thai<sup>34</sup>, *P.aeruginosa* bacteria can cause accelerating the microbial corrosion on aluminium alloy due to greater intensity of  $\text{Al}(\text{OH})_3$ ;  $(\text{Al}_2\text{O}_3)4\text{H}_2\text{O}$  that were observed on the test. Figure 4d,e,f showed the microstructure of the surface of alloy were covered with corrosion product due to presence of *P. fluorescens*, and Figure 4f showed the biofilm formed on the alloy surface clearly, this biofilm is non-uniform and heterogeneous on aluminum alloy and caused increasing in corrosion rate.



a) Control on Day 28 at Salinity of 33 ‰



d) Addition with 5% (v/v) of *P. flourescens* on Day 28 at Salinity of 33 ‰



b) Control on Day 28 at Salinity of 35‰



e) Addition with 5% (v/v) of *P. flourescens* on Day 28 at Salinity of 35‰

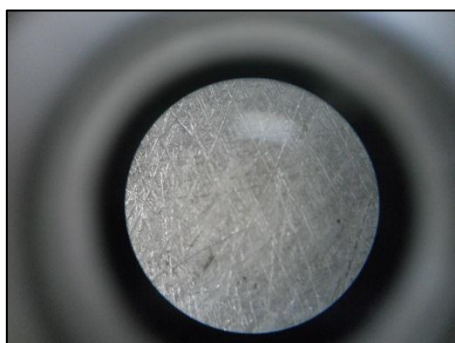


c) Control on Day 28 at Salinity of 37‰

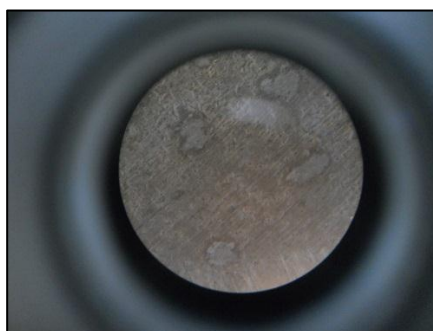


f) Addition with 5% (v/v) of *P. flourescens* on Day 28 at Salinity of 37‰

Figure 2. Visual observation on testing specimens

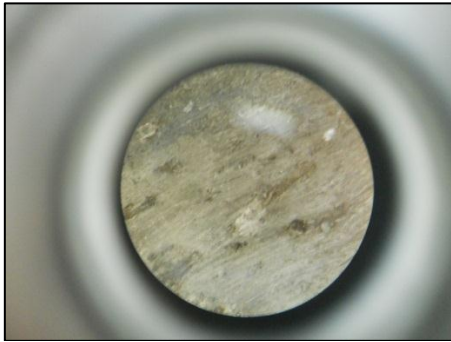


a) Control on Day 28 at Salinity of 33 ‰

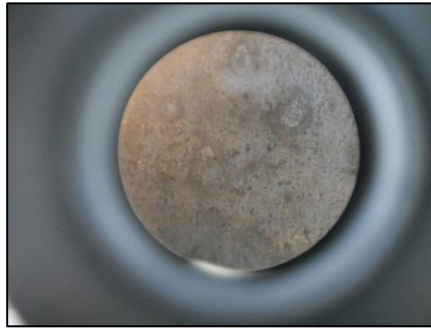


d) Addition with 5% (v/v) of *P. flourescens* on Day 28 at Salinity of 33 ‰

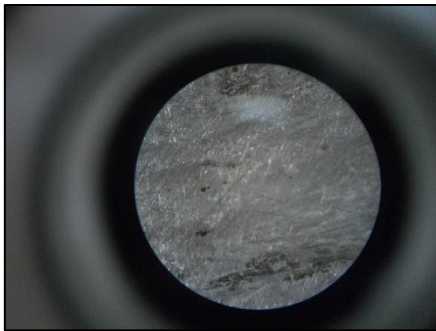




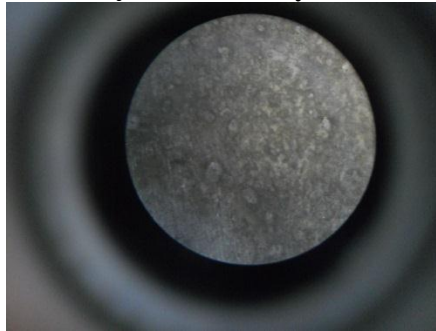
b) Control on Day 28 at Salinity of 35‰



e) Addition with 5% (v/v) of *P. fluorescens* on Day 28 at Salinity of 35‰

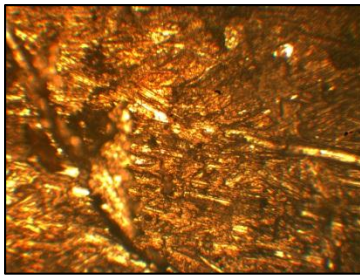


c) Control on Day 28 at Salinity of 37‰

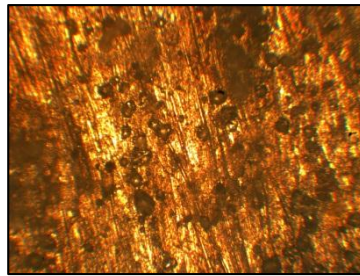


f) Addition with 5% (v/v) of *P. fluorescens* on Day 28 at Salinity of 37‰

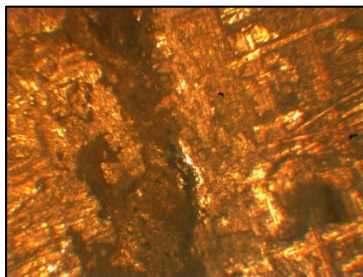
Figure 3. Macrostructure observation on testing specimens in magnification of 40X



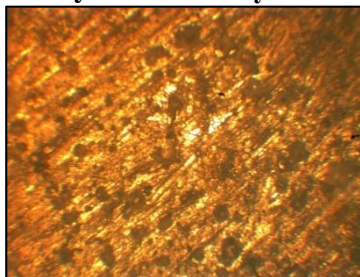
a) Control on Day 28 at Salinity of 33 ‰



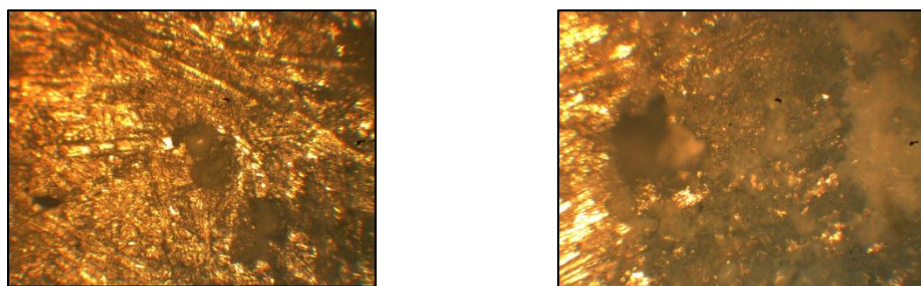
d) Addition with 5% (v/v) of *P. fluorescens* on Day 28 at Salinity of 33 ‰



b) Control on Day 28 at Salinity of 35‰



e) Addition with 5% (v/v) of *P. fluorescens* on Day 28 at Salinity of 35‰



c) Control on Day 28 at Salinity of 37‰ f) Addition with 5% (v/v) of *P. fluorescens* on Day 28 at Salinity of 37‰

**Figure 4. Microstructure observation on testing specimens in magnification of 100X**

## Conclusions

Based on the results, the higher salinity can cause heavy pitting and thus cause aluminium corrosion. The corrosion rate, without bacteria, on AA6063 after immersion in salinity of 33, 35 and 37‰ at day 28 were 0.5383, 0.6553 and 0.7021 mm/year, respectively. The MIC rate by *P. fluorescens* on AA 6063 at salinity of 37‰ was 1.1701 mm/year or increased by one point six-fold compared with the condition without bacteria addition at the same salinity of 37‰. In conclusion, *P. Fluorescens* has a role to increase of pitting corrosion rate on AA6063.

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